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LIST OF RELEVANT PAPERS

I changed my name from Line Nørregaard Olsen to Line Boel Nørregaard during the PhD period, which explains why my name appears differently in the authorship list.

Papers included in the thesis

- i. **Nørregaard, L. B.***, Wickham, K. A.*, Ehlers, T., Rocha, M. P., Fischer, M., Slingsby, M. H. L., Cheung, S. S., Evans, P. A., Bangsbo, J., & Hellsten, Y. (2023). Exercise training induces thrombogenic benefits in recent but not late postmenopausal females. *American Journal of Physiology: Heart and Circulatory Physiology*, 325(2), H346-H361.
- ii. **Nørregaard, L.B.***, Hansen, C.C.*, Wickham, K.A.*, Møller, S., Olsen, K., Ehlers, T., Bangsbo, J. & Hellsten Y. Exercise training alters skeletal muscle microvascular endothelial cell properties in recent postmenopausal females. Submitted, *Journal of Physiology*.
- iii. **Nørregaard, L.B.**, Wickham, K.A., Jeppesen, J., Rytter, N., Christoffersen L., Gliemann L., Lawrence, M., Evans P.A., Kruuse, C. & Hellsten, Y. (2023). Exercise transiently increases the density of incipient blood clots in antiplatelet-treated lacunar stroke patients. In review, *Thrombosis Research Journal*.

Published papers relevant for the thesis

- i. Wickham, K., **Nørregaard, L. B.**, Slingsby, M. H. L., Cheung, S. S., & Hellsten, Y. (2022). High-intensity exercise training improves basal platelet prostacyclin sensitivity and potentiates the response to dual anti-platelet therapy in postmenopausal women. *Biomolecules*, 12(10), [1501].
- ii. **Nørregaard Olsen, L.**, Høier, B., Hansen, C. V., Leinum, M., Carter, H. H., Jørgensen, T. S., Bangsbo, J., & Hellsten, Y. (2020). Angiogenic potential is reduced in skeletal muscle of aged women. *Journal of Physiology*, 598(22), 5149-5164.

- iii. Hoier B*, **Olsen LN***, Leinum M, Jørgensen TS, Carter HH, Hellsten Y and Bangsbo J (2021) Aerobic High-Intensity Exercise Training Improves Cardiovascular Health in Older Post-menopausal Women. *Front. Aging* 2:667519.
- iv. Wickham, K.A.*, **Nørregaard, L.B.***, Oxfeldt, M., Cheung, S.S., Gliemann, L., Hansen, M., and Hellsten, Y. (2021) Short-term Supplementation with Fermented Red Clover Extract Reduces Vascular Inflammation in Early Post-menopausal Women. *Front. Car Med.*

Reviews relevant for the thesis

- i. **Nørregaard Olsen, L*.**, Fischer, M*., Evans, P. A., Gliemann, L., & Hellsten, Y. (2021). Does exercise influence the susceptibility to arterial thrombosis? An integrative perspective. *Frontiers in Physiology*, 12, [636027].
- ii. Tamariz-Ellemann, A*., Wickham, K. A.*, **Nørregaard, L. B.**, Gliemann, L., & Hellsten, Y. (2023). The time is now: Regular exercise maintains vascular health in aging women. *Journal of Physiology*, 601(11), 2085-2098.

*Authors contributed equally.

ABSTRACT

Globally, cardiovascular disease is the leading cause of death, with stroke being the second most prevalent single cause. Endothelial dysfunction is a hallmark of cardiovascular disease ultimately increasing the risk of arterial thrombosis and leading to capillary rarefaction. Exercise plays an important role in both primary and secondary prevention of cardiovascular disease, however, acute exercise might increase the risk of thrombosis. The primary aim of this thesis was to evaluate the effect of aerobic exercise training and of acute exercise on thrombogenicity and factors affecting thrombogenicity in healthy and diseased cohorts. To address this aim we conducted one study in which healthy recent postmenopausal females (≤ 5 years post menopause) and late postmenopausal females (≥ 10 years post menopause) completed eight weeks of high-intensity exercise training and one study in which lacunar stroke patients and healthy elderly individuals completed one bout of acute exercise. The two studies allowed for an examination of the effect of exercise on the novel biomarker for clot microstructure, fractal dimension, and thereby the susceptibility to thrombosis. Focus in the study on the recent and late postmenopausal females was also on the impact of exercise training on properties of isolated muscle microvascular endothelial cells, vascular function and angiogenesis.

The main findings of this thesis were that eight weeks of high-intensity exercise training led to reduced clot microstructure and platelet reactivity, indicating a reduced susceptibility to thrombosis, in recent postmenopausal females (*Study I*). In microvascular endothelial cells derived from skeletal muscle biopsies of recent postmenopausal females, glycolysis increased and the production of reactive oxygen species (ROS) per respiration decreased with exercise training (*Study I*). Vascular function and skeletal muscle capillarization remained unaltered in both groups (*Study I*). Both lacunar stroke patients and healthy elderly individuals showed an increase in thrombogenicity in response to acute exercise, however at rest the thrombotic risk was higher in the stroke patients compared to the healthy elderly individuals (*Study II*).

In conclusion, this thesis shows for the first time, that late postmenopausal females present less adaptation to exercise training than recent postmenopausal females for many parameters, including thrombogenicity, and endothelial cell properties. It is therefore proposed that, for the best primary prevention, exercise training should be initiated soon after menopause. Additionally, our findings imply that exercise is important in the prevention of thrombotic risk. However, given the transient increase in thrombotic risk with acute exercise, for patients at risk, including lacunar stroke patients, exercise should be prescribed with caution to prevent a cardiovascular event following exercise.

DANISH SUMMARY

På verdensplan er hjertekarsygdomme den hyppigste dødsårsag. Endothel dysfunktion er et af kendetegnene ved hjertekarsygdomme, der både øger risikoen for arterielle blodpropper og fører til nedsat kapillærtæthed. Fysisk aktivitet spiller en vigtig rolle for både den primære og sekundære forebyggelse af hjertekarsygdomme, hvorimod et akut arbejde kan øge risikoen for blodpropper. Det primære formål med denne afhandling var at undersøge hvordan aerob træning og akut arbejde påvirker risikoen for blodpropper både i grupper af raske personer og patienter. Dette blev undersøgt i to studier. I det ene studie gennemførte kvinder få år (≤ 5 år) og kvinder mange år (≥ 10 år) efter overgangsalderen otte ugers højintensiv aerob træning, og i det andet studie gennemførte apopleksipatienter på blodpladehæmmende medicin og raske ældre personer et akut cykelarbejde. Disse to studier muliggjorde at kunne undersøge effekten af fysisk aktivitet på den strukturelle opbygning af en *in vitro* blodprop og dermed risikoen for udvikling af blodpropper. I studiet der inkluderede kvinder få år efter overgangsalderen og kvinder mange år efter overgangsalderen blev effekten af aerob træning på karakteristika af isolerede mikrovaskulære endothelceller, vaskulær funktion og dannelse af blodkar (angiogenese) også undersøgt.

Hovedresultaterne var at otte ugers højintens aerob træning reducerede densiteten af en *in vitro* blodprop og blodpladereaktiviteten hos kvinder få år efter overgangsalderen, hvilket indikerer en nedsat risiko for blodpropper (**Studie I**). I mikrovaskulære endothelceller udvundet fra skeletmuskelbiopsier hos kvinder få år efter overgangsalderen steg glykolysen og produktionen af frie radikaler per respiration faldt efter otte ugers højintens aerob træning (**Studie I**). Der var ingen effekt af otte ugers højintens aerob træning på den vaskulære funktion eller blodkardannelsen i skeletmuskulaturen hos nogen af grupperne (**Studie I**). Et akut cykelarbejde ledte til samme blodproppsfremmende effekt hos både apopleksipatienter og raske ældre individer, hvorimod risikoen for blodpropper i hvile var højere hos apopleksipatienterne sammenlignet med de raske ældre individer (**Studie II**).

Denne afhandling viste for første gang, eksperimentelt, at kvinder mange år efter overgangsalderen responderer mindre på højintens aerob træning sammenlignet med kvinder få år efter overgangsalderen i forhold til at opnå positive trombogener tilpasninger og forbedrede endothelcelle karakteristika. Fysisk aktivitet bør derfor påbegyndes kort efter overgangsalderens indtræden for at opnå den bedste primære forebyggelse af hjertekarsygdomme. Disse fund indikerer desuden at fysisk aktivitet er vigtig i forebyggelsen af blodpropper, men grundet den øgede risiko for blodpropper efter et akut arbejde, skal fysisk aktivitet tilbydes med varsomhed hos apopleksipatienter, for at forebygge akut hjertekarsygdom.